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COMMENTARY

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Further information on possible animal sources for human COVID-19

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Updated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) data in humans are provided in Table 1. This synopsis summarizes the latest findings on animal sources that could pose a risk for human SARS-CoV-2 infection and coronavirus disease-2019 (COVID-19). The information provided may be important during xenotransplantation or for immunocompromised individuals who own or work with animals on a regular basis. It is widely accepted that coronavirus species can be identified in both humans and various animal species and are commonly associated with respiratory or gastrointestinal disease, or both.[1] With SARS-CoV-2 cases in humans continuously increasing on a daily basis, it is important to understand which animal species may potentially be susceptible to SARS-CoV-2 infection and hence may serve as a reservoir for human infections.

Previously, it has been determined that animals within the *Felidae* (domestic cats; captive tigers and lions), *Canidae* (pet dogs), and *Mustelidae* (farmed minks) families can become naturally infected with SARS-CoV-2. The majority, if not all cases previously reported,[2] were due to the close contact of pets or farmed animals with COVID-19-infected patients. Table 2 provides updated information for *Felidae*, *Canidae* and *Mustelidae* but also additional species investigated. Previous results have been further confirmed in a recent study conducted in Northern Italy where more than 500 companion animals were sampled at the time of frequent human SARS-CoV2 infection.[3] While SARS-CoV-2 RNA was not found in any animal, 3.4% of all dogs and 3.9% of the cats investigated had measurable neutralizing antibody titers, furthermore, the presence of COVID-19 in a household was identified as a risk factor.[3] In addition, under experimental conditions, ferrets were shown to be a suitable model to mimic SARS-CoV-2 replication in the respiratory tract of humans. However, clinical signs or mortality are not always seen.[4] Moreover, a recent study indicated that SARS-CoV-2 is transmitted via direct contact within 1-3 days between ferrets housed in the same cage, but also via air within 3-7 days when housed in separate cages while sharing the same airflow.[5] The robust airborne transmission of SARS-CoV-2 shown in that study further confirms that physical distancing measures are important.[5]

Fortunately, SARS-CoV-2 experimental infection trials in poultry using chickens and ducks demonstrated a lack of susceptibility of these species to the virus (Table 2).[4,6] A recently published study with a larger cohort, conducted in the USA, further confirmed these negative results by expanding the poultry species range tested by also including turkeys, quails, and geese (Table 2).[7]

Since our last update, additional SARS-CoV-2-infected mink farms have been discovered with a total of 25 farms in the Netherlands[8,9] (https://promedmail.org/promed-post/?id=7588293), 3 farms in Denmark (https://www.oie.int/fileadmin/Home/MM/Update_1_Letter_to_OIE_about_the_COVID-19_situation_in_Denmark.pdf), and one farm in Spain (https://promedmail.org/promed-post/?id=7584560). Overall, one million Dutch minks and 100 000 Spanish minks have been culled so far (https://www.theguardian.com/world/2020/jul/17/spain-to-cull-nearly-100000-mink-in-coronavirus-outbreak). In general, affected mink farms are considered spillover events from the human pandemic and the source of infection was likely infected humans entering the farm.[8] However, humans infected by minks have also been identified[10] and this may have happened 2-6 times with the transmission route not entirely

TABLE 1 Facts on high pathogenic human CoVs

Virus	Time of circulation	Laboratory confirmed cases	Deaths	Case fatality rate (%)	Country distribution
SARS-CoV[a]	2002-2003	8096	774	9.6	26
MERS-CoV[b]	2012-ongoing	2494	853	35	27
SARS-CoV-2[c]	2019-ongoing	25 602 665	852 758	3.3	Global pandemic

[a]Source: https://www.who.int/csr/sars/country/table2004_04_21/en/.
[b]Source: https://www.who.int/emergencies/mers-cov/en/.
[c]Source: https://covid19.who.int (Accessed 2020/09/03).

established (https://www.government.nl/latest/news/2020/05/19/new-results-from-research-into-covid-19-on-mink-farms).

In addition to reports of more cases of naturally infected animals, new SARS-CoV-2 animal models have been reported (Table 2). Recently, clinical and pathological manifestations of COVID-19 have been reproduced in a golden Syrian hamster model.[11] Infected hamsters developed mild clinical signs and weight loss but eventually recovered and developed serum neutralizing antibodies 14 days post-challenge.[11] Similar results were also obtained by another group which demonstrated SARS-CoV-2 antigen by immunohistochemistry in nasal mucosa and bronchial epithelial cells between 2 and 5 days post-infection.[12] Since then, the golden Syrian hamster model has been used to show that surgical masks reduce the risk of SARS-CoV-2 contact transmission.[13] In fact, a surgical mask partition between challenged and naïve hamsters significantly reduced transmission to 25%.[13] Of note, mice, although members of the *Cricetiadae* family, are not susceptible to SARS-CoV-2 unless the virus is genetically adapted by serial passaging.[14] This perhaps indicates that a species cannot be categorized as susceptible or resistant due to their family.

Non-human primates (rhesus macaques) were successfully infected with SARS-CoV-2, and characteristic respiratory signs were observed in both 3- to 5-year-old and 15-year-old rhesus macaques (Table 2).[15] Viral replication in the respiratory tract was more pronounced in older monkeys and lasted for 14 days. These results confirm that rhesus macaques can be infected by SARS-CoV-2.[15] In line with this research, a US group used the rhesus macaque SARS-CoV-2 model to test protective immunity after re-exposure.[16] The rhesus macaques had high viral loads in the upper and lower respiratory tract and pathologic evidence of viral pneumonia after initial challenge. Following re-challenge, there was approximately a 5 $\log_{10}$ reduction in median SARS-CoV-2 viral loads in bronchoalveolar lavage and nasal mucosa samples when compared with viral loads after primary infection.[16] Similar results were also obtained by a Chinese group.[17] Furthermore, a SARS-CoV-2 DNA vaccine candidate was successfully tested in the rhesus macaque model indicating >3.1 $\log_{10}$ (bronchoalveolar lavage) and >3.7 $\log_{10}$ (nasal mucosa) reductions in median viral loads when compared to placebo controls.[18]

Several scientific groups have used an alternative approach to identify possible SARS-CoV-2 susceptible animals. Rather than searching for naturally infected animals or performing experimental infection trials, the receptor angiotensin-converting enzyme 2 (ACE2), which binds to the receptor binding domain (RBD) of the spike protein of SARS-CoV-2, essential for host cell entry and replication initialization, was investigated by comparing its structure across animal species. Early virus infectivity studies used HeLa cells that either expressed ACE2 proteins from selected species or not to show that SARS-CoV-2 uses ACE2 proteins for cell entry in humans, Chinese horseshoe bats, civets, and pigs, but not in mice.[19] In a follow-up study, X-ray structures of human ACE2 bound to the RBD of SARS-CoV-2 were used to predict its binding to ACE2 orthologue proteins from different animals.[20] Of the 20 amino acids in ACE2 that make contact with the spike protein, only 13 are necessary for ACE2 to function as a SARS-CoV-2 receptor, possibly indicating a minimal species barrier. Pigs and dogs were considered exceptions as they have low ACE2 expression in their respiratory tract.[20] Further, using flow cytometry to detect interactions of RBD-Fc proteins with ACE2 orthologues expressed on the surface of 293T cells, and assays with pseudoviruses expressing the spike protein, species with an orthologue ACE2 receptor were identified: ruminants (camels, cattle, goats, sheep), horses, pigs, cats, and rabbits; this receptor also supports viral entry of SARS-CoV-1, a bat-CoV (Bat-CoV RaTG13), and Pangolin-CoV.[21] Using a surface ACE2 binding assay with HeLa cells transduced with lentiviruses expressing ACE2 from different species, a different study investigated birds, reptiles (alligators, turtles, lizards), mammals, amphibians, coelacanths bone fish, and cartilaginous fish. ACE2 orthologues were identified in 80 mammalian species, including pets, livestock, and animals commonly found in zoos and aquaria.[22] Overall, results so far indicate that many more mammalian species may potentially be susceptible to SARS-CoV-2 infection and replication, and can therefore also serve as possible reservoirs.

New information recently became available on the possible origin of SARS-CoV-2. Soon after the discovery of SARS-CoV-2, bats had been suggested as the most likely reservoir host. As expected, 7/9 fruit bats (*Rousettus aegyptiacus*) had a transient SARS-CoV-2 infection after experimental inoculation and 1/3 contact bats also became infected.[6] Recently, the pangolin species has been suggested as a natural reservoir of SARS-CoV-2. Pangolin-associated coronaviruses belonging to two sub-lineages of SARS-CoV-2-related coronaviruses were identified in Malayan pangolins.[23,24] Specifically, five key amino acid residues of the RBD involved in the interaction with human ACE2 are consistent between Pangolin-CoV and SARS-CoV-2 in contrast to only one out of the five key residues between

TABLE 2 Summary of findings in animals to date (Adapted from OIE Technical Factsheet, Infection with SARS-CoV-2 in animals)

			Experimental infection characteristics			Susceptibility			Serological surveillance	
Family	Species	Type of infection	Animal# (Reference)	Route	Dose[a]	None, low, high	Clinical signs	Transmission	Positive/total number tested	Reference
Suidae	Pigs	Experimental	9^{6} 5^{4}	Intra-nasal	10^{5} $TCID_{50}$ 10^{5} PFU	None	No	No	0/187	26
Poultry	Chickens	Experimental	17^{6} 5^{4} 10^{7}	Oculo-oronasal Intra-nasal	10^{5} $TCID_{50}$ $10^{4.5}$ PFU $10^{5.4}$ $TCID_{50}$	None	No	No	0/153	26
	Duck	Experimental	5^{4} 10^{7}	Intra-nasal Intra-choanal	$10^{4.5}$ PFU 10^{6} $TCID_{50}$	None	No	No	0/153	26
	Turkeys	Experimental	10^{7}	Intra-choanal	$10^{5.4}$ $TCID_{50}$	None	No	No	NA	
	Japanese quail	Experimental	10^{7}	Intra-choanal	$10^{5.4}$ $TCID_{50}$	None	No	No	NA	
	White Chinese geese	Experimental	10^{7}	Intra-choanal	10^{6} $TCID_{50}$	None	No	No	NA	
Ruminants	Cattle	NA							0/107	26
	Sheep								0/133	26
	Goats								NA	
Caninae	Dogs	Natural and experimental	5^{4}	Intra-nasal	10^{5} PFU	Low	No or mild	No	8/180 0/497	3 26
Felidae	Cats (domestic)	Natural and experimental	14^{4} 3^{27}	Intra-nasal NA	10^{5} PFU NA	High	No or mild	Yes	6/60 0/87	3 26
	Tigers and lions	Natural				High	Yes	Yes	0/8	26
Musteliadae	Ferrets	Experimental	10^{6} 9^{4}	Intra-nasal	10^{5} $TCID_{50}$ 10^{5} PFU	High	No or mild	Yes	0/2	26
	Minks (American minks, *Neovison vison*)	Natural				High	Yes	Yes, also mink-human	0/81	26
Pteropodidae	Egyptian fruit bats (*Rousettus aegyptiacus*)	Experimental	9^{6}	Intra-nasal	10^{5} $TCID_{50}$	High	No	Yes	NA	
Cricetidae	Golden Syrian hamsters	Experimental	4^{5} 9^{12} 15^{11} 13^{13}	Intra-nasal	6×10^{5} $TCID_{50}$ 8×10^{4} $TCID_{50}$ 10^{5} PFU 10^{5} PFU	High	No or mild	Yes	NA	
Old word monkeys Subfamily *Cercopithecines*	Macaques (*Macaca fascicularis* and *Macaca mulatta*)	Experimental	5^{15} 3^{16} 3^{16} 3^{16} 10^{18}	Intra-nasal Intra-nasal and intra-tracheal	10^{6} $TCID_{50}$ 1.1×10^{6} PFU 1.1×10^{5} PFU 1.1×10^{4} PFU	High	Yes	Yes	NA	

Abbreviation: NA, not available.

[a]Median tissue culture infectious dose ($TCID_{50}$) per animal or plaque forming unit (PFU).

SARS-CoV-2 and Bat-CoV RaG13.[25] Moreover, at the whole genome level, Pangolin-CoV is 91.0% identical to SARS-CoV-2 whereas RaTG13 and Pangolin-CoV are only 90.6% identical.[25]

In summary, since SARS-CoV-2 emerged in the human population toward the end of 2019, it has been spreading at a high rate and infection rates in humans continue to increase. There is confirmed evidence that SARS-CoV-2 from COVID-19-infected humans can spillover to certain animal species within the families *Mustelidae*, *Felinae*, and *Caninae*. Commonly, infections in animal hosts are subclinical but occasionally clinical signs can be observed. Moreover, cats, dogs, ferrets, Egyptian fruit bats, golden Syrian hamsters, and macaques have been experimentally infected and some of these species are now used for SARS-CoV-2 research. There is however surprisingly little information on other species which are predicted to potentially serve as reservoirs for humans. Of note, the sample size of species that have been tested was low. This lack of knowledge requires attention, in cases of xenotransplantation most organs or products of animal origin should be tested for the presence of SARS-CoV-2 prior to their use in patients.

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